

WHAT IS CLAIMED IS:

- 1 1. A method of sialylating a saccharide group on a recombinant
2 glycoprotein, the method comprising contacting a saccharide group which comprises a
3 galactose or N-acetylgalactosamine acceptor moiety on a recombinant glycoprotein with a
4 sialic acid donor moiety and a recombinant sialyltransferase in a reaction mixture which
5 provides reactants required for sialyltransferase activity for a sufficient time and under
6 appropriate conditions to transfer sialic acid from said sialic acid donor moiety to said
7 saccharide group.
- 1 2. The method of claim 1, wherein the sialic acid donor moiety is CMP-
2 sialic acid.
- 1 3. The method of claim 2, wherein the CMP-sialic acid is enzymatically
2 generated *in situ*.
- 1 4. The method of claim 1, wherein the sialyltransferase is a recombinant
2 eukaryotic sialyltransferase which substantially lacks a membrane-spanning domain.
- 1 5. The method of claim 1, wherein the sialyltransferase includes a sialyl
2 motif which has an amino acid sequence that is at least about 40% identical to a sialyl motif
3 from a sialyltransferase selected from the group consisting of ST3Gal I, ST6Gal I, and
4 ST3Gal III.
- 1 6. The method of claim 1, wherein the sialyltransferase is a recombinant
2 ST3Gal III.
- 1 7. The method of claim 6, wherein the sialyltransferase is a recombinant
2 rat ST3Gal III.
- 1 8. The method of claim 1, wherein the sialyltransferase is a recombinant
2 ST3Gal IV.

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1 9. The method of claim 1, wherein the sialyltransferase is a recombinant
2 ST6Gal I.

1 10. The method of claim 1, wherein the sialyltransferase is a recombinant
2 ST3Gal I.

1 11. The method of claim 10, wherein the reaction mixture comprises a
2 second recombinant sialyltransferase, which second recombinant sialyltransferase is an
3 ST3Gal III.

1 12. The method of claim 1, wherein the sialyltransferase is a recombinant
2 bacterial sialyltransferase.

1 13. The method of claim 12, wherein the bacterial sialyltransferase has an
2 amino acid sequence which is at least 50% identical to an amino acid sequence of a *Neisseria*
3 *meningitidis* 2,3-sialyltransferase.

1 14. The method of claim 13, wherein the bacterial sialyltransferase is a
2 *Neisseria meningitidis* 2,3-sialyltransferase.

1 15. The method of claim 12, wherein the bacterial sialyltransferase has an
2 amino acid sequence which is at least 50% identical to an amino acid sequence of a
3 *Photobacterium damsela* 2,6-sialyltransferase.

1 16. The method of claim 15, wherein the bacterial sialyltransferase is a
2 *Photobacterium damsela* 2,6-sialyltransferase.

1 17. The method of claim 12, wherein the bacterial sialyltransferase has an
2 amino acid sequence which is at least 50% identical to an amino acid sequence of a
3 *Haemophilus* 2,3-sialyltransferase.

10081456-022102

1 18. The method of claim 17, wherein the sialyltransferase is a *Haemophilus*
2 2,3-sialyltransferase.

1 19. The method of claim 12, wherein the bacterial sialyltransferase has an
2 amino acid sequence which is at least 50% identical to an amino acid sequence of a
3 *Campylobacter jejuni* 2,3-sialyltransferase.

1 20. The method of claim 19, wherein the sialyltransferase is a
2 *Campylobacter jejuni* 2,3-sialyltransferase.

1 21. The method of claim 1, wherein the sialyltransferase is produced by
2 recombinant expression of a sialyltransferase in a host cell selected from the group
3 consisting of an insect cell, a mammalian cell, and a fungal cell.

1 22. The method of claim 21, wherein the host cell is an *Aspergillus niger*
2 cell.

1 23. A method of sialylating a saccharide group on a recombinant
2 glycoprotein, the method comprising contacting a saccharide group which comprises a
3 galactose or an N-acetylgalactosamine acceptor moiety on a recombinant glycoprotein with a
4 sialic acid donor moiety and a bacterial sialyltransferase in a reaction mixture which
5 provides reactants required for sialyltransferase activity for a sufficient time and under
6 appropriate conditions to transfer sialic acid from said sialic acid donor moiety to said
7 saccharide group.

1 24. The method of claim 23, wherein the bacterial sialyltransferase has an
2 amino acid sequence which is at least 50% identical to an amino acid sequence of a
3 *Photobacterium damsela* 2,6-sialyltransferase.

1 25. The method of claim 24, wherein the bacterial sialyltransferase is a
2 *Photobacterium damsela* 2,6-sialyltransferase.

10091456-022102

1 26. The method of claim 23, wherein the bacterial sialyltransferase has an
2 amino acid sequence which is at least 50% identical to an amino acid sequence of a *Neisseria*
3 *meningitidis* 2,3-sialyltransferase.

1 27. The method of claim 26, wherein the sialyltransferase is a *Neisseria*
2 *meningitidis* 2,3-sialyltransferase.

1 28. The method of claim 23, wherein the bacterial sialyltransferase has an
2 amino acid sequence which is at least 50% identical to an amino acid sequence of a
3 *Campylobacter jejuni* 2,3-sialyltransferase.

1 29. The method of claim 28, wherein the sialyltransferase is a
2 *Campylobacter jejuni* 2,3-sialyltransferase.

1 30. The method of claim 23, wherein the bacterial sialyltransferase has an
2 amino acid sequence which is at least 50% identical to an amino acid sequence of a
3 *Haemophilus* 2,3-sialyltransferase.

1 31. The method of claim 30, wherein the sialyltransferase is a *Haemophilus*
2 2,3-sialyltransferase.

1 32. A method for *in vitro* sialylation of saccharide groups present on a
2 glycoprotein, said method comprising contacting said saccharide groups with a
3 sialyltransferase, a sialic acid donor moiety, and other reactants required for sialyltransferase
4 activity for a sufficient time and under appropriate conditions to transfer sialic acid from said
5 sialic acid donor moiety to said saccharide group, wherein said sialyltransferase is present at
6 a concentration about 50 mU per mg of glycoprotein or less.

1 33. The method of claim 32, wherein the sialyltransferase is present at a
2 concentration of between about 5-25 mU per mg of glycoprotein.

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34. The method of claim 32, wherein the sialyltransferase is present at a concentration of between about 10-50 mU/ml of reaction mixture and the glycoprotein is present in the reaction mixture at a concentration of at least about 2 mg/ml.

35. The method of claim 32, wherein the method yields a glycoprotein having sialylation of at least about 80% of terminal galactose residues present on the saccharide groups.

36. The method of claim 32, wherein the sialyltransferase is a recombinant sialyltransferase.

37. The method of claim 36, wherein the sialyltransferase substantially lacks a membrane-spanning domain.

38. The method of claim 32, wherein the sialyltransferase includes a sialyl motif which has an amino acid sequence that is at least about 40% identical to a sialyl motif from a sialyltransferase selected from the group consisting of ST3Gal I, ST6Gal I, and ST3Gal III.

39. The method of claim 32, wherein the sialyltransferase is an ST3Gal III.

40. The method of claim 39, wherein the ST3Gal III is a rat ST3Gal III.

41. The method of claim 32, wherein the sialyltransferase is an ST3Gal IV.

42. The method of claim 32, wherein the sialyltransferase is an ST3Gal I.

43. The method of claim 42, wherein the reaction mixture comprises a second recombinant sialyltransferase, which second recombinant sialyltransferase is an ST3Gal III.

10081456-022102

1 44. The method of claim 32, wherein the sialyltransferase is a bacterial
2 sialyltransferase.

1 45. The method of claim 44, wherein the bacterial sialyltransferase is a
2 recombinant sialyltransferase.

1 46. The method of claim 44, wherein the bacterial sialyltransferase has an
2 amino acid sequence which is at least 50% identical to an amino acid sequence of a *Neisseria*
3 *meningitidis* 2,3-sialyltransferase.

1 47. The method of claim 46, wherein the bacterial sialyltransferase is a
2 *Neisseria meningitidis* 2,3-sialyltransferase.

1 48. The method of claim 44, wherein the bacterial sialyltransferase has an
2 amino acid sequence which is at least 50% identical to an amino acid sequence of a
3 *Photobacterium damsela* 2,6-sialyltransferase.

1 49. The method of claim 48, wherein the bacterial sialyltransferase is a
2 *Photobacterium damsela* 2,6-sialyltransferase.

1 50. The method of claim 44, wherein the bacterial sialyltransferase has an
2 amino acid sequence which is at least 50% identical to an amino acid sequence of a
3 *Campylobacter jejuni* 2,3-sialyltransferase.

1 51. The method of claim 50, wherein the sialyltransferase is a
2 *Campylobacter jejuni* 2,3-sialyltransferase.

1 52. The method of claim 44, wherein the bacterial sialyltransferase has an
2 amino acid sequence which is at least 50% identical to an amino acid sequence of a
3 *Haemophilus* 2,3-sialyltransferase.

1004456-022102

1 53. The method of claim 52, wherein the sialyltransferase is a *Haemophilus*
2 2,3-sialyltransferase.

1 54. The method of claim 32, wherein the sialic acid donor moiety is CMP-
2 sialic acid.

1 55. The method of claim 54, wherein the CMP-sialic acid is enzymatically
2 generated *in situ*.

1 56. The method of claim 32, wherein the sialic acid is selected from the
2 group consisting of NeuAc and NeuGc.

1 57. A method for *in vitro* sialylation of saccharide groups present on a
2 glycoprotein, the method comprising contacting the saccharide groups with an ST3Gal III
3 sialyltransferase, a sialic acid donor moiety, and other reactants required for sialyltransferase
4 activity for a sufficient time and under conditions to transfer sialic acid from said sialic acid
5 donor moiety to said saccharide group, wherein said ST3Gal III sialyltransferase is present at
6 a concentration of less than about 50 mU per mg of glycoprotein.

1 58. The method of claim 57, wherein the method further comprises
2 contacting the saccharide groups with an ST6GalII sialyltransferase.